Viral Oncogenesis in Tumors of the Central Nervous System: Reality or Random Association? A Retrospective Study on Archived Material

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Viral oncogenesis in tumors of the central nervous system: reality or random association? A retrospective study on archived material

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Abstract

Background: Central nervous system (CNS) tumors have devastating effects. They are recurrent, with dismal prognosis (gliomas) or life-threatening by compression effect (meningiomas). The etiology is still debatable, and over the last decade the hypothesis that human viruses may be implicated in these tumors has been proposed. Our aim was to examine the presence of eleven viruses in the most frequent CNS primary tumors. **Methods:** We assessed by PCR the viral presence in archived, paraffin embedded tumor tissues from 114 patients with glioma and meningioma and in the brain tissue from 40 controls lacking tumor pathology. We focused on candidate neuro-oncogenic types (herpesviridae and polyomaviruses) and on human papillomavirus (HPV). **Results:** HPV presence, for which an involvement in these tumors was hardly investigated, was found to be associated with both tumor categories compared with controls (glioma, $p=0.032$; meningioma, $p=0.032$), whereas the presence of the neuro-oncogenic viruses was found in a negligible number of both categories, suggesting a lack of association with the tumor presence. Moreover, our study revealed a positive correlation between HPV presence and glioma malignancy, and a negative correlation with meningioma grading. **Conclusions:** Our results suggest that the presence of HPV seems to be significantly associated with primary tumors of the CNS and its coverings.

Keywords: glioma; meningioma; HPV, polyomavirus, herpesvirus, oncogenesis

1. Background

Central nervous system (CNS) tumors account for a relatively small percentage of human tumors. However, since they cause morbidity and mortality that is disproportionate to their incidence, the mechanisms underpinning their etiopathogenesis are an important topic of research. There are over 100 different histological types of primary CNS tumors, with an annual age-adjusted incidence of 28.57 cases per 100.000 inhabitants. [1]

The most common tumors affecting the brain are brain metastases, whereas among primary tumors, gliomas (comprising glioblastomas, astrocytomas, oligodendrogliomas and ependymomas) are the most prevalent malignant types, while meningiomas are the most common mostly histologically benign tumors affecting the CNS. Glioblastomas constitute 45% of malignant primary brain tumors and, according to the Central Brain Tumor Registry of the United States data (2008-2012), and meningiomas account for 35% of all primary brain tumors [2].

The aggressiveness of glioblastomas is both histological and clinical, determined by their localization within a rigid space and adjacent to vital areas; as an example, patients with glioblastoma, the most aggressive glial primary tumor, have a 5 years survival rate of 5.1% and a median survival around 10 months [3]. Recent approaches for glioblastoma therapy address some pathogenic features and are based on immunological and genetic-mediated techniques in addition to classical surgery, radio and chemotherapies [4–6]. For meningiomas standard therapy consists of surgery with or without adjuvant radiation, depending on the tumor grade and the degree of resection [7].

As for any other tumor types, knowing the precise etiology and pathogenesis would be the essential step in order to treat, cure, or even prevent their onset. Little is known about the etiology of these brain proliferative conditions and currently the only established risk factors are represented by ionizing radiation and some rare genetic related syndromes [8]. Moreover, there is growing evidence and consensus for the association of certain viruses with brain tumors [9]. Even though some viruses have been recently proposed as a therapeutic method for aggressive glial tumors [10–13], the viral role as an etiological agent is not sufficiently understood.

Altogether, approximately 12% of human cancers has been supposed to have a viral etiology [14]. Human viral oncogenesis has been suggested to be characterized by three main features: a) oncoviruses are necessary, but not sufficient for the development of cancer; b) virus-dependent cancers occur many years after acute infection and after persistent chronic infections and; c) the immune system can act both in the direction of immunosuppression and chronic inflammation. [15, 16]. Viruses activate various intracellular metabolic pathways, with the progressive accumulation of cellular markers that constitute the malignant phenotype. This is achieved by the

accumulation of somatic oncogenic alterations (oncogenic strokes), caused by spontaneous mutations or mutations induced by exposure to environmental carcinogens, in the context of a genetic background of the host and the selective pressure imposed by the tissue microenvironment [17]. The viral oncogenic activity is most recently considered as oncomodulatory [18]. Several types of viral infection have been suggested to be potentially associated with CNS tumors, especially glioblastomas. Herpesviridae is a large family of DNA viruses that comprises Herpes Simplex 1 and 2 (HHV1, HHV2), Varicella zoster virus (HHV-3), Epstein–Barr virus (EBV or HHV-4), Cytomegalovirus (CMV or HHV-5), Roseolovirus (HHV-6 and HHV7) and Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8). These neurotropic viruses in the CNS are responsible for the activation of glial cells that release pro-inflammatory mediators and increase oxidative stress which are associated with neurodegenerative diseases pathogenesis. Indeed, the presence of many of these viruses have been related to Alzheimer disease [19], Parkinson [20] and epilepsy [21].

Regarding cancer, although EBV and KSHV, are officially recognized as carcinogens, also the other viruses from this family have been associated with different types of cancer, but their role in carcinogenesis remains unclear [22].

Emerging evidence demonstrated the presence of human CMV proteins and nucleic acids in brain tumors in both adults and children [23], detecting the virus presence particularly in patients with high-grade gliomas (Ranganathan et al. 2012; Goerig et al. 2017). However, data are contrasting and the need for research into the involvement of this virus in gliomas has recently been argued [25, 26] since the CMV presence seems to be limited only to glioblastoma and not present in other types of glial tumors. [27, 28] Moreover, the real presence of CMV in gliomas is questioned [29, 30] and single cell sequencing does not clearly highlight the presence of this virus [31]

Also the results regarding the role of Epstein-Barr virus (HHV-4) in glioma genesis are controversial [32]. Although its presence was detected with a higher frequency than other herpesviruses (HHV-5, HHV-6, HHV-8) in pilocytic astrocytoma, it was not considered as being responsible for tumorigenesis in those cases [33].

Also HHV-6 is potentially involved in glial oncogenesis. The ability of HHV-6 to integrate into a chromosomal region that is highly relevant to carcinogenesis and the ability of HHV-6 ORF-1 protein to bind to p53 and its detection of early and late antigens in adult primary and recurrent CNS tumors (more frequently in glial tumors) [34] make this virus a potential key actor in glioma cases [35]. However, further studies are considered as mandatory [36].

HHV-8 or Kaposi's sarcoma-associated herpesvirus is well known for its oncogenic role in a wide category of haematological malignancies. However, since it has also a strong neurotropism [37], it could modulate the proliferation of glioma stem cells [38].

Moreover, HHV-2, EBV and HHV-6 have been detected in high-grade glioma tissues, and HHV-1 and HHV-2 immunoreactivity was found in some cases, although these were not detected in their tumor tissue [39].

Human polyomaviruses represent a diverse group of human pathogens comprising John Cunningham virus (JCV), BK virus and simian virus 40 (SV40) that generally cause asymptomatic infection in healthy individuals. JC and BK viruses are described as being associated mostly with cutaneous and mucosal tumors, as well as with bladder carcinoma [40]. These viruses have been involved in neural oncogenesis, mainly in glioblastoma [39]. In particular, glioblastoma with small cell neuronal-like component was mainly related to JCV [41]. However, the role of this virus in carcinogenesis of brain tumors is not completely understood [42, 43].

Regarding SV40, most studies on its presence were performed in the sera of glioma patients, without assessing its presence within the brain tissue [44]. Moreover, differences regarding the detection methods (viral DNA, mRNA, expression of viral oncoproteins) are considered as essential in affirming or denying SV40 involvement as an oncogenic agent [45]. Finally, a potential involvement of HPV in glioblastoma has been suggested [28] since this virus has been found to have a prognostic implications in glioblastomas [46].

2. Methods

In this retrospective study we aim to detect the presence of various potential oncogenic viruses in the most frequent tumors of the brain and its coverings (glioblastoma and meningioma) compared to patients lacking tumor pathology.

2.1 Sample collection

In this study we enrolled 154 individuals. Fifty-six patients have been diagnosed with meningioma, and 58 with glioma (44 glioblastoma, 9 grade two diffuse astrocytoma and 5 anaplastic astrocytoma). Forty patients represented the control group, deceased by various pathologies and lacking any brain tumor. All study specimens have been collected from the Neuropathology Department of the National Institute of Neurology and Neurovascular Diseases. The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the National Institute of Pathology "Victor Babes" (Registration number

53 of 6th December 2017). All the patients or their relatives signed the written informed consent. Only patients with primary tumors of the brain and meninges have been enrolled. We excluded metastases (with biology specific to the tissue of origin), glandular (pineal, pituitary) or accessory tissue (eye) tumors.

Serial 5- μ m sections from formalin-fixed, paraffin-embedded (FFPE) tissues were cut from each paraffin block. The first section was stained with Hematoxylin–Eosin and a second histopathological diagnosis was established by an expert pathologist, in order to confirm the initial classification of the cases. All tumors were classified and graded according to the WHO classification of CNS tumors [47]. Samples with a little amount of tissue, presenting large necrotic area or presenting artefacts (i.e. intraoperative electrical mark), detected by microscopic examination, have been excluded.

The initial series comprised all primary tumor categories of the brain, including oligodendroglioma, ependymoma, choroid plexus tumors, etc, with a total of 200 cases. However, since most of these other entities were represented by a too small number of cases, we restricted our research only to the most common and aggressive brain tumor, the grade IV glioblastoma.

The sociodemographic and clinical data of the selected cohorts are reported in Table 1.

Table1: Sociodemographic and clinical data of controls and patients involved in the study.

	Controls C (N=40)	Meningioma M (N=56)	Glioma G (N=58)		<i>p-value</i>
			Glioblastoma GB Grade IV (N=44)	Astrocytoma A Grade II and III (N=14)	
Sex F (N, %)	19 (47.5%)	36 (64.3%)	15(34.1%)	9(64.3%)	<i>M vs C</i> p=0.101; $\chi^2=2.687$ <i>G vs C</i> p=0.548; $\chi^2=0.360$ <i>GB vs C</i> p=0.243; $\chi^2=1.364$ <i>A vs C</i> p=0.279; $\chi^2=1.170$
Age±SD	65.55±15.63	57.52±12.78	58.27±10.87	47.29±14.28	<i>M vs C</i> p= 0.007 <i>G vs C</i> p= 0.001 <i>GB vs C</i> p= 0.017 <i>A vs C</i> p< 0.001
Localization (N)	Cortex=25	Convexity=35	Temporal=16	Frontal=6	
	Striatum=4	Basal=21	Frontal=11	Temporal=4	
	Hippocampus=3		Parieto-occipital=5	Cerebellum=1	
	Mesencephalon=3		Parietal=3	Fronto-bilateral=1	
	Hippocampus=3		Temporo-parietal=3	Fronto-temporal=1	
	Cerebellum=2		Fronto-temporal=2	Temporal-lateral ventricle=1	
	Cingulate gyrus=1		Cerebellum=1		
	Pons=1		Fronto-parietal=1		
	White matter=1		Temporal-lateral ventricle=1		
			Third ventricle=1		
Grading		Grade I, benign=38			
		Grade II, atypical=15		Grade II, diffuse astrocytoma=9	
		Grade III, malignant=3		Grade III, anaplastic astrocytoma=5	

2.2 DNA isolation, virus detection and HPV genotyping.

A number of 5–10 slides (50 µm) were cut from each sample, according to the volume. Before the DNA isolation, in order to remove the paraffin, sections were placed at room temperature in two baths of xylene and absolute ethanol. Genomic DNA from FFPE tissue sections was isolated using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. The quantity and purity of DNA were determined using the NanoDrop 2000 (Thermo Scientific, DE, USA).

The presence of HHV1 and HHV2, HHV4-5 and BK, HHV6, HHV7, HHV8, JCV and SV40 viruses have been detected using dedicated Genesig Advanced kits (Primerdesign Ltd., York House, School Lane, Chandler's Ford, United Kingdom) by qPCR, following the instructions of the manufacturer on the ABI7500 fast (Applied Biosystem, MA, USA). Each kit comprises a positive control that contains templates for the targets in the test, and endogenous control to confirm extraction of a valid biological template.

The presence of HPV has been detected by PCR using consensus primers targeting L1 region, GP5+/GP6+ primer set [48] as described by Yoshida et al [49]. This generated a 140-bp-long fragment from the HPV L1 structural gene. The amplified DNA was subjected to electrophoresis on 2% agarose gel, and then visualized by ultraviolet illumination using ethidium bromide. PCR reactions that were negative after first round, were subjected to a further 30 rounds of PCR amplification using the same primers (auto nested PCR)

For samples found HPV positive by auto-nested PCR, genotyping has been performed using the AMPLIQUALITY HPV-TYPE EXPRESS v3.0 (AB Analitica, Padua, Italy) that allows the identification of the presence of HPV as well as the detection of 40 HPV genotypes: HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68 (68a e 68b), 69, 70, 71, 72, 73, 81, 82, 83, 84, 87, 89, 90. This kit is IVD (In Vitro Diagnostic) marked for the detection of HPV also in histological FFPE samples. With this method, we did not obtained PCR products after the first amplification. Thus, PCR reactions were subjected to a further 30 rounds of PCR amplification (auto nested PCR)

2.3 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS version 17.0). Continuous variable (Age) was tested with the t-test. For categorical variables, frequency with percentages was reported and assessed for the two groups by χ^2 test if the number of patients in a subgroup was ≥ 5 , and when the number was less than 5 in any cell, the Fisher exact test was applied.

3. Results

A total of 154 individuals have been involved in the study, including 79 female (51.3%) and 75 male (48.7%). The mean age of the study population was 58.89 years (SD ± 14.01). Three groups have been made according to the diagnosis of meningioma (n=56), glioma (n=58, including 44 glioblastoma and 14 astrocytoma) and control (n=40). The groups were homogenous for sex, but not for age, since the control group was older than the groups

of patients ($p < 0.05$). Regarding the grading, in the meningioma group most of the tumors were of grade I (67.86%) followed by grade II (26.78%) and only 5.36% were of grade III. The glioma group comprised the 75.86% of glioblastoma, grade IV and the 24.14% of astrocytoma. Among these, 64.28% were diffuse astrocytoma (grade II) and 35.72% anaplastic astrocytoma (grade III).

The presence of HPV was detected in the 20.78% of 154 analyzed samples with 3 positive cases in the control group, 14 in the meningioma group and 15 in the glioma group (13 in the glioblastoma and 2 in the astrocytoma subgroup).

The HPV presence was more frequent in patients with glioma compared to the control group with a significance of $p = 0.032$ (Fig 1a) as well as in the glioblastoma subgroup compared with controls ($p = 0.012$) (Fig 1b). No statistical difference was found comparing the astrocytoma group vs control. A statistical significance was observed also comparing meningioma vs control group ($p = 0.032$) (Fig 2a). In the meningioma group the presence of HPV was more frequent in meningioma of grade I, benign (12 positive cases out of 38, 31.6%) than in grade II, atypical (2 positive cases out of 15, 13.3%) and grade III, malignant where no positive cases has been detected on the three cases (Fig 2b).

Figure 1: Percentage of positive HPV cases in control and glioma groups: **a)** comparison between controls and patients with glioma **b)** distribution of HPV positive cases in glioma groups stratified in astrocytoma and glioblastoma.

Figure 2: Percentage of positive HPV cases in control and meningioma groups: **a)** comparison between controls and patients with meningioma **b)** distribution of HPV positive cases in meningioma group stratified by grading

Regarding the localization no statistical difference has been observed between convexity meningioma (9 HPV positive cases out of 35) and basal meningioma (5 HPV positive cases out of 21). (Additional file 1: Table. S1)

The presence of HPV in the positive cases has been validated using an IVD kit. However, none of the 40 tested genotypes was found in our groups.

An analysis looking at the HPV frequency in age categories (Age $\leq$ 60 and Age $>$ 60) has been conducted in the entire selected cohort and in the three groups. No statistical difference has been found between the two age categories in any analyzed group (Tab. 2).

Table 2: Distribution of HPV positive cases in age categories in the different analyzed groups

	All individuals (N=154)		Controls (N=40)		Meningioma (N=56)		Glioma (N=58)	
	Age $\leq$ 60	Age $>$ 60	Age $\leq$ 60	Age $>$ 60	Age $\leq$ 60	Age $>$ 60	Age $\leq$ 60	Age $>$ 60
HPV -	62	60	16	21	21	21	25	18
HPV+	14	18	0	3	8	6	6	9
	$\chi^2=0.507$ p=0.476		Fisher p=0.262		$\chi^2=0.215$ p=0.643		$\chi^2=1.471$ p=0.225	

The distribution of HPV positive and negative cases in more stringent age categories is reported in (Additional file 1: Table S2).

The presence of HPV in sex categories was also investigated finding that in the entire cohort (N=154) the number of HPV positive cases was higher in the female group (26.6%) than in the male group (14.7%), however without

statistical significance ($\chi^2=3.318$, $p=0.069$). This trend of difference between male and female was observed also in the control, glioma and meningioma groups, again without statistical significance. (Additional file 1: Table S3)

HHV4-EBV was identified in 4.5% of total samples (3 positive cases in the control group, 1 in the meningioma group, 1 in the glioblastoma subgroup and 2 in the astrocytoma subgroup). No statistical difference regarding the presence of this virus among the groups was found. One of the positive patients with glioblastoma has co-infection with HPV and another with HHV6.

HHV6 was detected in the same percentage as HHV4, but with a different distribution among the groups, with 4 positive cases in the control group, 1 in the meningioma group, 1 in the glioblastoma subgroup and 1 in the astrocytoma subgroup. Again, no statistical difference among the groups was shown. Of note, the patient with glioblastoma HHV6 positive presented co-infection with HPV.

The presence of HHV7 was detected only in one patient with meningioma with HPV co-infection, whereas the presence of HHV1-2, HHV5-CMV, HHV8, JCV, BKV, SV40 was not detected in the control group or in the patient group. All the results are shown in Table 3.

Table 3: Presence of all the analyzed viruses in controls, patients with meningioma and glioma

	Controls C (N=40)	Meningioma M (N=56)	Glioma G (N=58)		<i>p-value</i>
			Glioblastoma GB (N=44)	Astrocytoma A (N=14)	
HHV1-2 (N+, %)	0	0	0	0	--
HHV4-EBV (N+, %)	3 (7.5%)	1 (1.78%)	1(2.3%)	2(14.3%)	<i>M vs C</i> p=0.305 <i>G vs C</i> p=0.686 <i>GB vs C</i> p=0.343 <i>A vs C</i> p=0.595
HHV5-CMV (N+, %)	0	0	0	0	--
HHV6 (N+, %)	4 (10%)	1 (1.78%)	1(2.3%)	1(7.1%)	<i>M vs C</i> p=0.157 <i>G vs C</i> p=0.222 <i>GB vs C</i> p=0.187 <i>A vs C</i> p>0.999
HHV7 (N+, %)	0	1 (1.78%)	0	0	--
HHV8 (N+, %)	0	0	0	0	--
JCV (N+, %)	0	0	0	0	--
BKV (N+, %)	0	0	0	0	--
SV40 (N+, %)	0	0	0	0	--
HPV (N+, %)	3 (7.5%)	14 (25%)	13(29.5%)	2(14.3%)	<i>M vs C</i> p=0.032 <i>G vs C</i> p=0.032 <i>GB vs C</i> p=0.012 <i>A vs C</i> p=0.595

4. Discussion

In this study we explored the presence of infectious agents belonging to the Herpesviridae family (HHV-1, HHV-2, HHV4-EBV, HHV5-CMV, HHV6, HHV7 and HHV8), polyomaviruses JCV, BKV and SV40 and HPV in patients with the most common brain tumors (glioma and meningioma) compared to a group of controls. We found that the most frequent virus in our analyzed cohort (40 controls, 56 meningiomas and 58 gliomas) was represented by HPV with 20.78% of positive cases. Specifically, HPV was detected in 25% of meningioma cases, 25.86% of glioma cases and in the 7.5% of control samples. Hence, HPV DNA presence was statistically more frequent in patients with glioma and meningioma compared to the controls ($p=0.032$ and $p=0.032$, respectively). Of note, the HPV positivity in the glioma group was more present in the glioblastoma subgroup (29.5%) compared to controls ($p=0.012$).

A striking phenomenon is the negative correlation between the HPV positivity and meningioma grading, with the highest frequency of positive cases in grade I meningioma (31.6%), followed by grade II (13.3%) and no cases in grade III (malignant) tumors. This could be due to genetic abnormalities specific to each meningioma group (benign, atypical, malignant), which could interfere with the viral infection. Since the spectrum of histological types of meningeal tumors is remarkably wide, ranging from epithelioid cells, to obviously mesenchymal ones (as is the fibrous variant), such particular interactions between the cell of origin (the arachnoid cap cell) and HPV is supposed to generate mostly benign categories of meningotheelial proliferations.

No statistical association between the other selected viruses and the analyzed brain tumors was observed in our study.

Our results on HPV in glioma and glioblastoma are in line with those reported by Vidone and collaborators [46] and by the research groups of Hashida [28] and Adnan [50] which found 25-28% HPV positive cases in patients with glioblastoma, emphasizing the possible role of HPV in the pathophysiological mechanism of this tumor. Because of the positive correlation between HPV presence and glioma malignancy observed in our study (14.3% in grade II and III astrocytoma and 29.5% in glioblastoma), we could speculate that HPV acts as a trigger for malignant transformation of normal glial cells: more cells infected generate a more intense proliferation index, increasing the tumor aggressiveness.

Again, in line with our findings, the Hashida study did not show a correlation between CMV as well as HHV-8 or EBV presence with glioblastoma [28]. In our study, the HPV DNA detection has been performed using two methods (in house PCR and a commercial IVD kit). None of the 40 HPV genotypes available in the kit was detected in our positive samples. This could mean that other HPV genotypes are involved in cerebral oncogenesis, or that the IVD kit, designed for detecting HPV in other tissues, such as mucosa or epidermis, are not adequate for processing brain tissue. In both procedures (in house PCR and IVD kit) the virus presence was detectable only after auto-nested PCR. This can be due to the low amounts of viral HPV DNA copies in brain tissues or to the tissue processing with large periods of formalin fixation.

Regarding viruses belonging to herpesviridae family, our results showing the lack of association between CMV and brain tumors are in line with what found by Holdhoff and collaborators [27], although in others studies CMV, as well as other herpesviridae including EBV have been detected in glioblastoma and other gliomas [51, 52]. Of note, some of these studies have been performed without control cases, leading to a partial conclusion about the oncogenic role of these viruses in glioma and glioblastoma [53]. The lack of positivity for HHV-8 (even in the

control tissue) disagrees with other studies, which affirm its positivity in 63.3% of healthy postmortem brain tissue [37]. In our study cohort, HHV-6 was more frequently detected in low-grade glioma (7.1%) almost as in control cases (10%), compared to glioblastoma (2.3%) or meningioma (1.78%). This is in agreement with other studies which found a higher presence of this HHV type in lower grade gliomas [54].

Herpes viruses, mainly HHV-6, followed by HHV-1, HHV-3, EBV, HHV-2 and CMV, are present in relatively high percentages in the facial or trigeminal ganglia in the general population [55] and their role in oncogenesis has not been established. The peripheral presence of such infection does not mean that the infection propagate to the profound structures of the nervous system. The trajectory of the viral agent could be indeed stopped by the microenvironment conditions of the peripheral ganglia. This mechanism may represent an explanation of the low frequency of HHV positive cases detected in our entire cohort.

Finally, in our cohort the presence of polyomaviruses (JC, BK or SV40) was not detected. Data from literature affirm that in adulthood a large percentage of population have been infected with JC and BK viruses. Indeed, a sero prevalence of 39% for JC and 82% for BK was detected in other studies, with the profile of young subjects being similar to adults, demonstrating that the primary exposure occurs precociously in life [56]. However, these data derive from seric determinations and the presence of these polyomaviruses in the brain tissue was not determined in parallel. Hence, the viruses could be latent only in circulating mononuclear blood cells or the cells of the proximal renal tubule, reactivating in immunocompromised individuals.

5. Conclusions

Taken together our findings, even though based on a rather small amount of cases, show a significant increased frequency of HPV in glioblastoma and meningioma compared to normal brain tissue and suggest the non-involvement of herpes and polyomaviruses in the investigated brain tumors. However, the detection of a virus within the tumor tissue is not sufficient to affirm its role as a causative agent. Further studies on larger cohorts are needed to clarify the role of these infectious agents in brain tumors in order to develop new preventive or therapeutic strategies.

List of abbreviations: BK, BK polyomavirus; CNS, central nervous system, CMV, cytomegalovirus; EBV, Epstein-Barr virus; FFPE, formalin-fixed, paraffin-embedded; HHV, human herpes virus; HPV, human

papilloma virus; IVD, in vitro diagnostic; JCV, John Cunningham virus; KSHV, Kaposi's sarcoma-associated herpesvirus; SV40, Simian virus 40.

Declarations

Ethics approval and consent to participate: the study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the National Institute of Pathology “Victor Babes” (Registration number 53 of 6th December 2017). All the individuals involved in the study or their relatives signed the written informed consent.

Consent for publication: not applicable.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its additional information files.

Competing interests: the authors declare that they have no competing interests.

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Author Contributions: DEA coordinated the study, was responsible of the collection and diagnosis of the samples included in the study, revised the manuscript and gave the final approval of the version to be published. MD designed the study, performed virus detection, contributed to data processing and writing. EM was responsible for the statistical analysis and wrote the first draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C, et al. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. *Neuro Oncol.* 2015;17 Suppl 4:iv1-iv62. doi:10.1093/neuonc/nov189.

338 2. McFaline-Figueroa JR, Lee EQ. Brain Tumors. *Am J Med.* 2018;131:874–82.
339 doi:10.1016/j.amjmed.2017.12.039.

340 3. Taylor OG, Brzozowski JS, Skelding KA. Glioblastoma Multiforme: An Overview of Emerging Therapeutic
341 Targets. *Front Oncol.* 2019;9:963. doi:10.3389/fonc.2019.00963.

342 4. Wheeler LA, Manzanera AG, Bell SD, Cavaliere R, McGregor JM, Grecula JC, et al. Phase II multicenter
343 study of gene-mediated cytotoxic immunotherapy as adjuvant to surgical resection for newly diagnosed
344 malignant glioma. *Neuro Oncol.* 2016;18:1137–45. doi:10.1093/neuonc/now002.

345 5. Kieran MW, Goumnerova L, Manley P, Chi SN, Marcus KJ, Manzanera AG, et al. Phase I study of gene-
346 mediated cytotoxic immunotherapy with AdV-tk as adjuvant to surgery and radiation for pediatric malignant
347 glioma and recurrent ependymoma. *Neuro Oncol.* 2019;21:537–46. doi:10.1093/neuonc/noy202.

348 6. Sait SF, Karajannis MA. The new kid on the block: suicide gene therapy to modulate cancer
349 immunosurveillance for children with high-risk malignant brain tumors. *Neuro Oncol.* 2019;21:419–20.
350 doi:10.1093/neuonc/noz026.

351 7. Wang N, Osswald M. Meningiomas: Overview and New Directions in Therapy. *Semin Neurol.* 2018;38:112–
352 20. doi:10.1055/s-0038-1636502.

353 8. Perkins A, Liu G. Primary Brain Tumors in Adults: Diagnosis and Treatment. *Am Fam Physician.*
354 2016;93:211–7. <http://www.ncbi.nlm.nih.gov/pubmed/26926614>.

355 9. Cai Z, Yang S, Li X, Chen F, Li W. Viral infection and glioma: a meta-analysis of prognosis. *BMC Cancer.*
356 2020;20:549. doi:10.1186/s12885-020-06796-3.

357 10. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michielin O, et al. Oncolytic
358 Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. *Cell.*
359 2017;170:1109–1119.e10. doi:10.1016/j.cell.2017.08.027.

360 11. Peruzzi P, Chiocca EA. Viruses in cancer therapy - from benchwarmers to quarterbacks. *Nat Rev Clin*
361 *Oncol.* 2018;15:657–8. doi:10.1038/s41571-018-0077-0.

362 12. Lang FF, Conrad C, Gomez-Manzano C, Yung WKA, Sawaya R, Weinberg JS, et al. Phase I Study of
363 DNX-2401 (Delta-24-RGD) Oncolytic Adenovirus: Replication and Immunotherapeutic Effects in Recurrent
364 Malignant Glioma. *J Clin Oncol.* 2018;36:1419–27. doi:10.1200/JCO.2017.75.8219.

365 13. Iorgulescu JB, Reardon DA, Chiocca EA, Wu CJ. Immunotherapy for glioblastoma: going viral. *Nat Med.*
366 2018;24:1094–6. doi:10.1038/s41591-018-0142-3.

367 14. Mesri EA, Feitelson MA, Munger K. Human viral oncogenesis: a cancer hallmarks analysis. *Cell Host*
368 *Microbe.* 2014;15:266–82. doi:10.1016/j.chom.2014.02.011.

369 15. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--
370 Part B: biological agents. *Lancet Oncol.* 2009;10:321–2. doi:10.1016/s1470-2045(09)70096-8.

371 16. zur Hausen H. The search for infectious causes of human cancers: where and why (Nobel lecture). *Angew*
372 *Chem Int Ed Engl.* 2009;48:5798–808. doi:10.1002/anie.200901917.

373 17. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144:646–74.
374 doi:10.1016/j.cell.2011.02.013.

375 18. Blaylock RL. Accelerated cancer aggressiveness by viral oncomodulation: New targets and newer natural
376 treatments for cancer control and treatment. *Surg Neurol Int.* 2019;10:199. doi:10.25259/SNI_361_2019.

377 19. Sochocka M, Zwolińska K, Leszek J. The Infectious Etiology of Alzheimer's Disease. *Curr*
378 *Neuropharmacol.* 2017;15:996–1009. doi:10.2174/1570159X15666170313122937.

379 20. Hemling N, Røyttä M, Rinne J, Pöllänen P, Broberg E, Tapio V, et al. Herpesviruses in brains in
380 Alzheimer's and Parkinson's diseases. *Ann Neurol.* 2003;54:267–71. doi:10.1002/ana.10662.

381 21. Karatas H, Gurer G, Pinar A, Soylemezoglu F, Tezel GG, Hascelik G, et al. Investigation of HSV-1, HSV-2,
382 CMV, HHV-6 and HHV-8 DNA by real-time PCR in surgical resection materials of epilepsy patients with
383 mesial temporal lobe sclerosis. *J Neurol Sci.* 2008;264:151–6. doi:10.1016/j.jns.2007.08.010.

384 22. Alibek K, Baiken Y, Kakpenova A, Mussabekova A, Zhussupbekova S, Akan M, et al. Implication of
385 human herpesviruses in oncogenesis through immune evasion and suppression. *Infect Agent Cancer.* 2014;9:3.
386 doi:10.1186/1750-9378-9-3.

387 23. Söderberg-Nauclér C, Johnsen JJ. Cytomegalovirus infection in brain tumors: A potential new target for
388 therapy? *Oncoimmunology.* 2012;1:739–40. doi:10.4161/onci.19441.

389 24. Goerig NL, Frey B, Überla K, Gaipl U, Fietkau R. A clinician's plea to test glioma patients for CMV. *Neuro*
390 *Oncol.* 2017;19:1282–3. doi:10.1093/neuonc/nox080.

25. Cobbs CS. Cytomegalovirus and brain tumor: epidemiology, biology and therapeutic aspects. *Curr Opin Oncol.* 2013;25:682–8. doi:10.1097/CCO.0000000000000005.
26. Johnson KJ, Hainfellner JA, Lau CC, Scheurer ME, Woehrer A, Wiemels J. Immune factors and viral interactions in brain cancer etiology and outcomes, The 2016 Brain Tumor Epidemiology Consortium Meeting report. *Clin Neuropathol.* 35:280–6. doi:10.5414/NP300985.
27. Holdhoff M, Guner G, Rodriguez FJ, Hicks JL, Zheng Q, Forman MS, et al. Absence of Cytomegalovirus in Glioblastoma and Other High-grade Gliomas by Real-time PCR, Immunohistochemistry, and In Situ Hybridization. *Clin Cancer Res.* 2017;23:3150–7. doi:10.1158/1078-0432.CCR-16-1490.
28. Hashida Y, Taniguchi A, Yawata T, Hosokawa S, Murakami M, Hiroi M, et al. Prevalence of human cytomegalovirus, polyomaviruses, and oncogenic viruses in glioblastoma among Japanese subjects. *Infect Agent Cancer.* 2015;10:3. doi:10.1186/1750-9378-10-3.
29. Wick W, Platten M. CMV infection and glioma, a highly controversial concept struggling in the clinical arena. *Neuro Oncol.* 2014;16:332–3. doi:10.1093/neuonc/nou002.
30. Rahman M, Dastmalchi F, Karachi A, Mitchell D. The role of CMV in glioblastoma and implications for immunotherapeutic strategies. *Oncoimmunology.* 2019;8:e1514921. doi:10.1080/2162402X.2018.1514921.
31. Johnson TS, Abrams ZB, Mo X, Zhang Y, Huang K. Lack of human cytomegalovirus expression in single cells from glioblastoma tumors and cell lines. *J Neurovirol.* 2017;23:671–8. doi:10.1007/s13365-017-0543-y.
32. Akhtar S, Vranic S, Cyprian FS, Al Moustafa A-E. Epstein-Barr Virus in Gliomas: Cause, Association, or Artifact? *Front Oncol.* 2018;8:123. doi:10.3389/fonc.2018.00123.
33. Neves AM, Thompson G, Carvalheira J, Trindade JC, Rueff J, Caetano JM, et al. Detection and quantitative analysis of human herpesvirus in pilocytic astrocytoma. *Brain Res.* 2008;1221:108–14. doi:10.1016/j.brainres.2008.05.009.
34. Crawford JR, Santi MR, Cornelison R, Sallinen S-L, Haapasalo H, MacDonald TJ. Detection of human herpesvirus-6 in adult central nervous system tumors: predominance of early and late viral antigens in glial tumors. *J Neurooncol.* 2009;95:49–60. doi:10.1007/s11060-009-9908-2.
35. Amirian ES, Scheurer ME. Chromosomally-integrated human herpesvirus 6 in familial glioma etiology. *Med Hypotheses.* 2012;79:193–6. doi:10.1016/j.mehy.2012.04.033.

418 36. Eliassen E, Lum E, Pritchett J, Ongradi J, Krueger G, Crawford JR, et al. Human Herpesvirus 6 and
419 Malignancy: A Review. *Front Oncol.* 2018;8:512. doi:10.3389/fonc.2018.00512.

420 37. Chan PK, Ng HK, Cheung JL, Cheng AF. Survey for the presence and distribution of human herpesvirus 8
421 in healthy brain. *J Clin Microbiol.* 2000;38:2772–3. doi:10.1128/JCM.38.7.2772-2773.2000.

422 38. Jeon H, Kang YH, Yoo S-M, Park M-J, Park JB, Lee S-H, et al. Kaposi's Sarcoma-Associated Herpesvirus
423 Infection Modulates the Proliferation of Glioma Stem-Like Cells. *J Microbiol Biotechnol.* 2018;28:165–74.
424 doi:10.4014/jmb.1709.09001.

425 39. Strojnik T, Duh D, Lah TT. Prevalence of Neurotropic Viruses in Malignant Glioma and Their Onco-
426 Modulatory Potential. *In Vivo.* 31:221–9. doi:10.21873/invivo.11049.

427 40. Møllerup S, Asplund M, Friis-Nielsen J, Kjartansdóttir KR, Fridholm H, Hansen TA, et al. High-
428 Throughput Sequencing-Based Investigation of Viruses in Human Cancers by Multienrichment Approach. *J*
429 *Infect Dis.* 2019;220:1312–24. doi:10.1093/infdis/jiz318.

430 41. Piña-Oviedo S, De León-Bojorge B, Cuesta-Mejías T, White MK, Ortiz-Hidalgo C, Khalili K, et al.
431 Glioblastoma multiforme with small cell neuronal-like component: association with human neurotropic JC
432 virus. *Acta Neuropathol.* 2006;111:388–96. doi:10.1007/s00401-006-0050-3.

433 42. Eftimov T, Enchev Y, Tsekov I, Simeonov P, Kalvatchev Z, Encheva E. JC polyomavirus in the aetiology
434 and pathophysiology of glial tumours. *Neurosurg Rev.* 2016;39:47–53. doi:10.1007/s10143-015-0676-5.

435 43. Delbue S, Comar M, Ferrante P. Review on the role of the human Polyomavirus JC in the development of
436 tumors. *Infect Agent Cancer.* 2017;12:10. doi:10.1186/s13027-017-0122-0.

437 44. Mazzoni E, Gerosa M, Lupidi F, Corallini A, Taronna AP, D'Agostino A, et al. Significant prevalence of
438 antibodies reacting with simian virus 40 mimotopes in sera from patients affected by glioblastoma multiforme.
439 *Neuro Oncol.* 2014;16:513–9. doi:10.1093/neuonc/not217.

440 45. Rotondo JC, Mazzoni E, Bononi I, Tognon M, Martini F. Association Between Simian Virus 40 and Human
441 Tumors. *Front Oncol.* 2019;9:670. doi:10.3389/fonc.2019.00670.

442 46. Vidone M, Alessandrini F, Marucci G, Farnedi A, de Biase D, Ricceri F, et al. Evidence of association of
443 human papillomavirus with prognosis worsening in glioblastoma multiforme. *Neuro Oncol.* 2014;16:298–302.
444 doi:10.1093/neuonc/not140.

445 47. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016
446 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta*
447 *Neuropathol.* 2016;131:803–20. doi:10.1007/s00401-016-1545-1.

448 48. Remmerbach TW, Brinckmann UG, Hemprich A, Chekol M, Kühndel K, Liebert UG. PCR detection of
449 human papillomavirus of the mucosa: comparison between MY09/11 and GP5+/6+ primer sets. *J Clin Virol.*
450 2004;30:302–8. doi:10.1016/j.jcv.2003.12.011.

451 49. Yoshida H, Murono S, Ueno T, Nakanishi Y, Tsuji A, Hatano M, et al. Usefulness of human papillomavirus
452 detection in oral rinse as a biomarker of oropharyngeal cancer. *Acta Otolaryngol.* 2017;137:773–7.
453 doi:10.1080/00016489.2016.1274426.

454 50. Adnan Ali SM, Mirza Y, Ahmad Z, Zahid N, Enam SA. Human Papillomavirus and Human
455 Cytomegalovirus Infection and Association with Prognosis in Patients with Primary Glioblastoma in Pakistan.
456 *World Neurosurg.* 2019;121:e931–9. doi:10.1016/j.wneu.2018.10.018.

457 51. Zavala-Vega S, Castro-Escarpulli G, Hernández-Santos H, Salinas-Lara C, Palma I, Mejía-Aranguré JM, et
458 al. An overview of the infection of CMV, HSV 1/2 and EBV in Mexican patients with glioblastoma multiforme.
459 *Pathol Res Pract.* 2017;213:271–6. doi:10.1016/j.prp.2016.12.006.

460 52. Fonseca RF, Rosas SLB, Oliveira JA, Teixeira A, Alves G, Carvalho M da GC. Frequency of Epstein-Barr
461 virus DNA sequences in human gliomas. *Sao Paulo Med J.* 2015;133:51–4. doi:10.1590/1516-
462 3180.2013.1912814.

463 53. McFaline-Figueroa JR, Wen PY. The Viral Connection to Glioblastoma. *Curr Infect Dis Rep.* 2017;19:5.
464 doi:10.1007/s11908-017-0563-z.

465 54. Crawford JR, Santi MR, Thorarinsdottir HK, Cornelison R, Rushing EJ, Zhang H, et al. Detection of human
466 herpesvirus-6 variants in pediatric brain tumors: association of viral antigen in low grade gliomas. *J Clin Virol.*
467 2009;46:37–42. doi:10.1016/j.jcv.2009.05.011.

468 55. Ptasińska-Sarosiek I, Dunaj J, Zajkowska A, Niemcunowicz-Janica A, Król M, Pancewicz S, et al. Post-
469 mortem detection of six human herpesviruses (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6) in trigeminal and
470 facial nerve ganglia by PCR. *PeerJ.* 2019;6:e6095. doi:10.7717/peerj.6095.

471 56. Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. *PLoS Pathog.*

472 2009;5:e1000363. doi:10.1371/journal.ppat.1000363.

Figures

(a)

(b)

Figure 1

Percentage of positive HPV cases in control and glioma groups: a) comparison between controls and patients with glioma b) distribution of HPV positive cases in glioma groups stratified in astrocytoma and glioblastoma.

(a)

(b)

Figure 2

Percentage of positive HPV cases in control and meningioma groups: a) comparison between controls and patients with meningioma b) distribution of HPV positive cases in meningioma group stratified by grading

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